Pathogenesis of cytomegalovirus-associated pneumonitis in ICR mice: possible involvement of superoxide radicals

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Summary. We have studied the pathogenesis of murine cytomegalovirus (MCMV) pneumonitis in immunocompetent ICR mice and in mice treated with cyclophosphamide (CP). Intranasal infection of immunocompetent mice with MCMV resulted in transient and self-limited pulmonary lesions. When mice were given 200 mg/kg of CP one day before virus infection, transient splenic atrophy and subsequent splenic hypertrophy were induced, and the lesions in the lung were markedly augmented in their number and size although there was no significant enhancement of the virus growth. The augmentation coincided with the period of splenic hypertrophy. A marked increase in the number of pulmonary lesions was also induced in mice given 100 mg/kg of CP every 4 days following the initial dose of 200 mg/kg. In these mice, however, continuous splenic atrophy and augmented replication of MCMV in the lung were observed. When the activity of xanthine oxidase (XO) in lung tissue homogenates was measured, the activity was found to significantly increase after intranasal infection with MCMV irrespective of CP administration and there was a good correlation between the elevation of XO activity and the degree of pathological changes in the lung. In addition, we found that the administration of allopurinol, a specific inhibitor of XO and superoxide dismutase, a superoxide radical scavenger, reduced the number of the pulmonary lesions. These results suggest that superoxide radicals are involved in the pathogenesis of MCMV-associated pneumonitis in ICR mice.

Introduction

Infection with human cytomegalovirus (HCMV) is an important cause of morbidity and mortality in severely immunocompromised patients, particularly in

those who have had allogeneic bone marrow or solid organ transplantation, or who have the acquired immunodeficiency syndrome (AIDS), and HCMV-associated interstitial pneumonitis is now a major cause of death in those patients. However, the pathogenesis of HCMV pneumonitis is not completely understood. On clinical setting, graft-versus-host disease (GVHD) is often associated with HCMV-associated interstitial pneumonitis after bone marrow transplantation; no cases of HCMV pneumonitis were found among 100 recipients of syngeneic bone marrow from identical twins in which no GVHD occurred [2]. HCMV pneumonitis after allogeneic bone marrow transplantation have not been successfully treated with ganciclovir (9-[2-hydroxy-1-(hydroxy-methyl)ethoxymethyl]guanine) alone despite its excellent antiviral activity, but better therapeutic effects of ganciclovir were obtained when the drug was given in combination with anti-HCMV immunoglobulin [10, 23]. In murine models of CMV pneumonitis, immunosuppression is often required to produce pathological changes [6]. However, immunosuppression is not mandatory [16, 22, 25]. On the contrary, immunosuppression inhibited the formation of pulmonary lesions in an animal model [27]. From these studies, it is suggested that CMV pneumonitis is an immunopathological condition [13]. Similar observations have been made in murine models of influenza virus pneumonitis. It has been suggested that the lethal effect of influenza infection is determined by immunopathological consequences of the host rather than the direct cytopathic effect of viral replication [14]. Furthermore, recent studies have shown that the enhanced production of superoxide radicals by xanthine oxidase (XO) plays a key role in the pathogenesis of influenza virus-induced pulmonary infection [1, 19].

In this study, we have established a model of murine cytomegalovirus (MCMV) pneumonitis in ICR mice, measured XO activity in lung tissues, and evaluated the effect of superoxide dismutase (SOD) and allopurinol in this model. The degree of the pathological changes expressed as the number of foci correlated well with XO activity but not with virus titers, and both SOD and allopurinol administration suppressed the formation of pulmonary lesions. We suggest that superoxide radicals generated by XO are involved in the pathogenesis of MCMV-associated pneumonitis in mice.

Materials and methods

Mice

Specific pathogen free male ICR mice (4-weeks-old) were purchased from Japan SLC (Shizuoka, Japan) and used throughout this study. Infected mice were maintained in groups of 3 to 6 in isolator units, and given water and food (Oriental Yeast Co., Ltd., Japan) ad libitum.

Virus

The Smith strain of MCMV has been kindly provided by Dr. Y. Minamishima (Department of Microbiology, Miyazaki Medical College, Miyazaki, Japan). The virus was maintained

by serial passage in ICR mice and was harvested as a 10% (wt/vol) homogenate of salivary gland tissue. Since it has been reported that such a salivary gland tissue homogenate itself induces a non-specific bronchiolitis [16, 27], virus stocks were prepared from tissue culture supernatants after a single passage in mouse embryo fibroblasts. The stocks, which usually contained $1 \sim 2 \times 10^6$ PFU of MCMV per ml, were stored at $-70\,°C$ until use.

Intranasal inoculation of virus

For infection mice were anesthetized with 200 mg/kg body weight of 2,2,2-tribromoethanol (Avertin; Aldrich Chemical Co., Milwaukee, WI, U.S.A.) and 0.05 ml of virus stock $(7 \times 10^4$ PFU) was instilled into the nose. Control mice were inoculated in parallel with an equivalent volume of culture medium.

Quantitation of virus

Lungs and other organs were excised from lethally anesthetized mice. Homogenates of these organs (10% wt/vol) were prepared in Eagle's minimal essential medium (MEM) containing 2% fetal calf serum (FCS) and were stored at $-70\,°C$ until virus assay. Mouse embryo fibroblasts were prepared from embryos of late-term pregnant ICR mice by trypsinization, and were maintained in MEM containing 10% FCS. Infectivity of MCMV was titrated by plaquing on secondary mouse embryo fibroblasts in duplicate 35 mm dishes (Falcon Labware, Becton-Dickinson & Co., Oxnard, CA, U.S.A.). After a 1 h adsorption period at 37 °C, the cultures were overlaid with 2 ml of 0.5% agarose in MEM containing 2% FCS. The monolayers were fixed with 5% formalin 4 days after infection and stained with crystal violet, and the number of plaques were counted by using a dissecting microscope at × 20 magnification.

Cyclophosphamide administration

Cyclophosphamide (CP) (Sigma Chemical Co., St. Louis, MO, U.S.A.) was prepared as a 20 mg/ml solution in phosphate-buffered saline (PBS) and given intraperitoneally.

Xanthine oxidase activity

Lung tissues excised from mice were immediately homogenized with 5 ml of ice-cold PBS containing 2 mM EDTA, 2 mM phenylmethanesulfonyl fluoride (PMSF), and 10 mM dithiothreitol (DTT) in Potter-Elvehjem homogenizer. The homogenates were centrifuged at 10,000 g for 10 min at 4 °C. The supernatants were assayed for XO activity according to the method described by Avis et al. [3]. The conversion of xanthine to uric acid was followed at wave length of 295 nm in a 1 ml reaction volume containing 0.1 mM xanthine (Katayama Chemical Co., Osaka, Japan) in 0.01 M PBS, pH 7.4 for 20 min at 25 °C. One unit of enzyme activity is defined as the amount of enzyme that caused the formation of 1 μmol of uric acid per minute using an extinction coefficient difference at 295 nm of $9.6 \times 10^3\,cm^{-1}\,M^{-1}$ [17].

Histology

For histologic evaluation of tissues, lungs were fixed with 10% buffered formalin. Lungs and hearts were taken en bloc from lethally anesthetized animals and were perfused with the buffered formalin via the pulmonary artery. Then the lungs were gently inflated by intratracheal instillation of the fixative. The paraffin-embedded sections were stained with hematoxylin and eosin (H & E). To estimate the magnitude of pathologic changes, inflammatory foci per section were counted under light microscope at × 40 magnification.

Immunohistochemistry

Some of the sections were immunohistochemically analyzed using a monoclonal antibody to a nuclear antigen of MCMV-infected cells, as described previously [32]. This monoclonal antibody, when tested in cultured mouse fibroblasts, reacted with the nuclear antigen 2 h after infection and also reacted with nuclear inclusions in a late phase of infection. The sections were treated with 0.15% periodic acid in distilled water at room temperature for 15 min to inactivate endogenous peroxidase, washed with PBS three times, and then incubated with the monoclonal antibody at room temperature for 45 min. After washing with PBS, the sections were incubated with peroxidase-conjugated antimouse IgG (Cappel Lab., Cochranville, PA, U.S.A.) for 45 min at room temperature. Then, the diaminobenzidine reaction was performed as described previously [32].

Administration of SOD and allopurinol

Recombinant human SOD was provided by Research Laboratories, Pharmaceutical Group, Nippon Kayaku Co., Ltd., Tokyo, Japan. SOD was prepared as a 20 kU/ml solution in normal saline. Mice were intravenously given 2,000 U/body of SOD every 12 h. Allopurinol (Tanabe Seiyaku Co., Ltd., Osaka, Japan) was prepared as a 10 mg/ml solution in normal saline. Mice were intraperitoneally given 2 mg/body of allopurinol every 24 h. Control mice were treated with an equivalent volume of vehicle (normal saline).

Statistics

Values are expressed as mean ± standard error (SE). Student's t-test was used for statistical analysis. Significant difference is defined as $p < 0.05$.

Results

Establishment and characterization of MCMV pneumonitis in ICR mice

Replication of MCMV and pathological changes in the lung of normal mice

Experiments were performed to determine the susceptibility of ICR mice to MCMV infection. Mice were intranasally infected with MCMV Smith strain at a dose of 7×10^4 PFU. Viruses were readily recovered from a number of major organs including spleen, liver and salivary gland (data not shown), but mortality was not observed. As shown in Fig. 1 B, panel a, virus titers in lung tissues already reached the plateau by day 3, and decreased gradually after day 10, but significant amounts of infectious virus were detectable even after 20 days after infection.

Light microscopic observation of lung tissues showed focal peribronchiolar mononuclear cell infiltration with occasional extension into the interstitium of alveolar septae. These inflammatory foci first appeared on day 3, reached the maximum in number between day 6 and day 10, and then disappeared in spite of the presence of substantial titers of MCMV (Fig. 1 C, panel a).

Effect of cyclophosphamide administration

In order to modulate the immune system, we used two kinds of CP administration regimens similar to that described by Shanley et al. [27]; one group of

mice (CP 1 mice) was administrated 200 mg/kg of CP one day before virus inoculation and the other group of mice (CPn mice) was administrated 100 mg/kg of CP every four days after an initial dose of 200 mg/kg one day before virus infection. As reported previously [27], a single dose of CP (200 mg/kg) resulted in marked splenic atrophy on day 3 but induced marked hypertrophy on day 10 (Fig. 1 A, panel b). There was no significant difference in the virus growth between MCMV-infected mice and MCMV-infected CP 1 mice (Fig. 1 B, panels a and b). However, a single dose of CP before MCMV infection resulted in a marked increase in the number of foci in the lung (Fig. 1 C, panel b). No mortality was seen. On the other hand, MCMV-infected CPn mice exhibited a marked increase in MCMV titers in the lung on and after day 6 (Fig. 1 B, panel c). In this regimen of CP administration spleen remained atrophic (Fig. 1 A, panel c). About a half of mice were killed by day 15 and mortality reached 100% by day 20. The number of foci on day 6 in MCMV-infected CPn mice was similar to that of mice infected with MCMV alone, but markedly increased thereafter (Fig. 1 C, panels a and c). It was also noted that there was a marked increase in the lung index (lung weight/body weight ratio) (Fig. 1 D, panel c).

Histopathology of the lung

Lungs were taken from mice for histological examination on day 3, 6, 10, 15 and 20. Neither tissue damage nor MCMV-antigen positive cells were seen in the tissues from mock-infected mice (Fig. 2 a) or CP-treated mock-infected mice. Inflammatory foci were detectable on day 3 in all of the three groups of MCMV-infected mice irrespective of CP administration. As described above, the number of foci in the lung was much larger in MCMV-infected CP 1 mice and MCMV-infected CPn mice than in MCMV-infected mice. There was essentially no histological difference between MCMV-infected CP 1 mice and MCMV-infected mice, except that the sizes of the foci in MCMV-infected CP 1 mice were larger than those of MCMV-infected mice. The lesions consisted of peribronchiolar mononuclear cell infiltration with nuclear debris and fibrin (Fig. 2 b and c). In the foci, a few MCMV-antigen positive cells were detected by immunohisto-chemistry (Fig. 2 d), but there were few MCMV-antigen positive cells in the other areas of the lung.

MCMV-infected CPn mice showed different histopathological abnormalities. The number of MCMV-antigen positive cells in the foci was significantly larger in MCMV-infected CPn mice than in MCMV-infected mice or MCMV-infected CP 1 mice (Fig. 3 b). The foci consisted of much less cellular components and much more fibrin when compared with those in MCMV-infected CP 1 mice (Figs. 2 c and 3 a). In MCMV-infected CPn mice, MCMV antigens were frequently detected in alveolar macrophages, bronchial epithelial cells (Fig. 3 c) and vascular endothelial cells (Fig. 3 d).

Activity of xanthine oxidase (XO)

In order to elucidate the role of superoxide radicals in the pathogenesis of MCMV-associated pneumonitis, we measured XO activity in the lung tissue
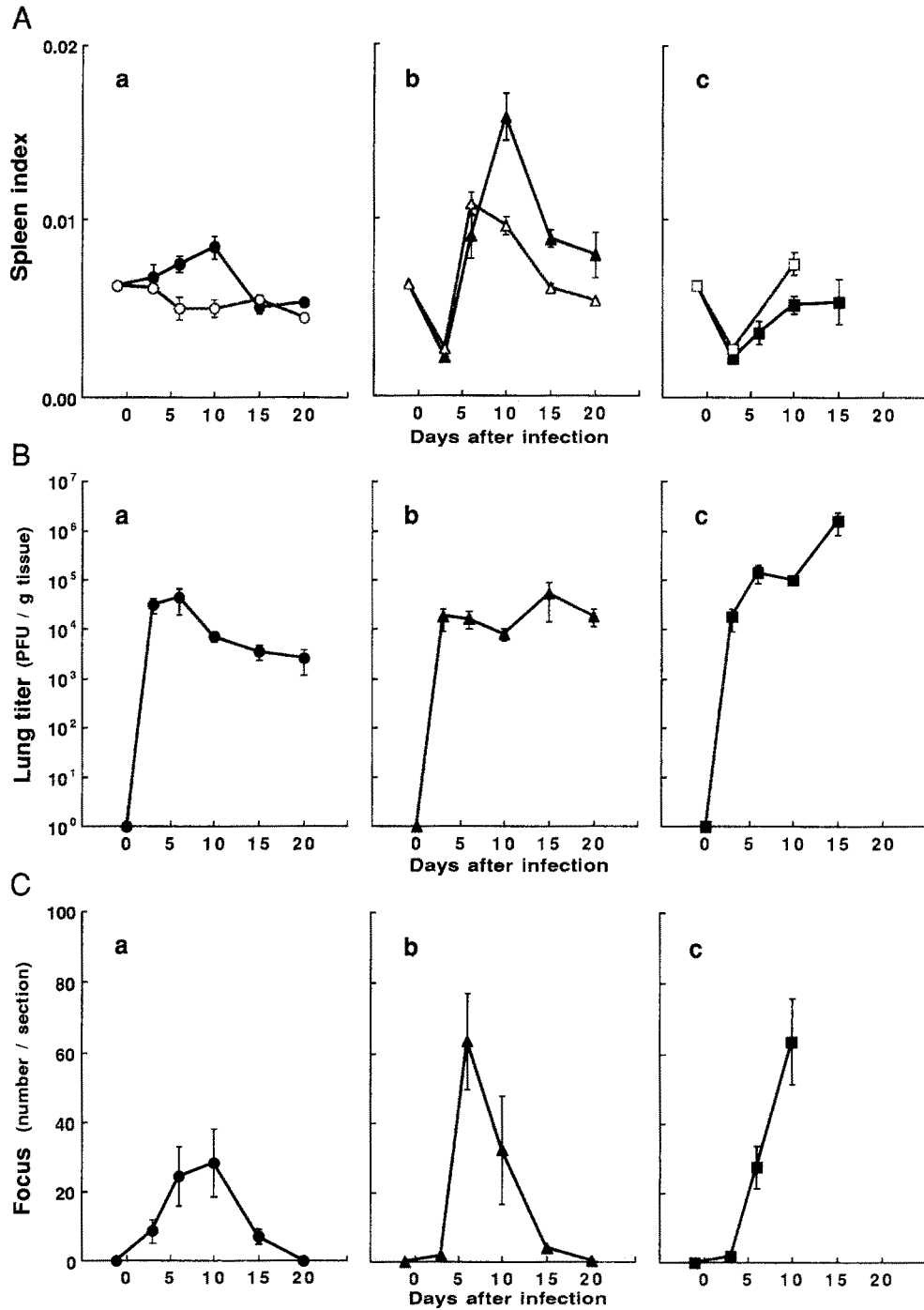
Fig. 1. Characteristics of lung infection with MCMV in ICR mice. Mice were intranasally inoculated with 7×10^4 PFU of MCMV on day 0. At appropriate times after infection, mice were sacrificed and examined on **A** spleen index, spleen weight/body weight ratio, **B** titers of MCMV in the lung, **C** number of pathological foci per section, **D** lung index, lung weight (mg)/body weight (g) ratio, and **E** XO activity in lung tissue homogenates. *a* MCMV-infected mice (●); *b* MCMV-infected CP 1 mice (▲); *c* MCMV-infected CPn mice (■). The results of mock-infected mice (○), mock-infected CP 1 mice (△), and mock-infected CPn mice (□) are also shown. Each point represents mean ± standard error (SE) in three animals

D

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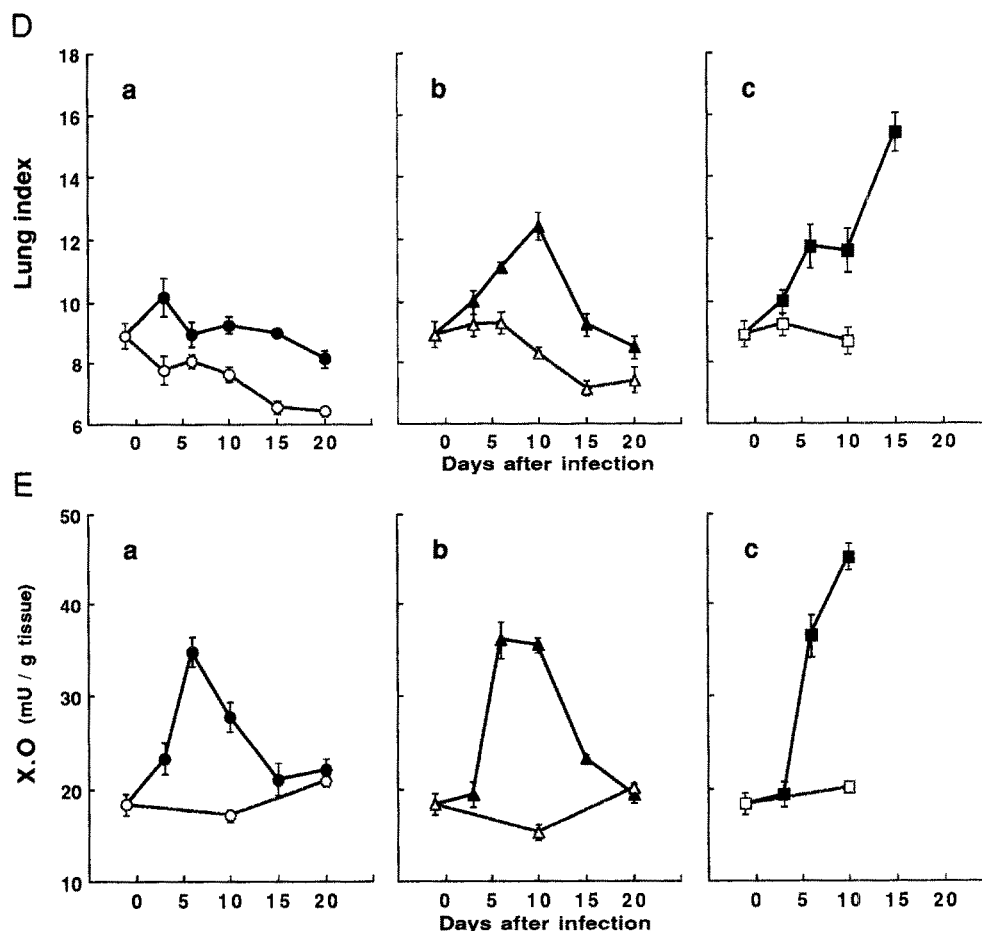
homogenates. As shown in Fig. 1 E, panel a, intranasal infection with MCMV in normal mice resulted in an increase in the level of XO activity. The activity reached the maximum level (34.8 ± 1.6 mU/g tissue) on day 6, and then rapidly decreased. In MCMV-infected CP 1 mice, however, the increase in XO activity persisted until day 10 although the overall time-course of this elevation of XO activity was similar to that of MCMV-infected mice (Fig. 1 E, panel b). In MCMV-infected CPn mice, XO activity continued to increase until day 10, and the activity (45.2 ± 1.5 mU/g tissue) on day 10 was significantly higher than that of MCMV-infected mice (27.6 ± 1.6 mU/g tissue) or MCMV-infected CP 1 mice (35.5 ± 0.8 mU/g tissue) (Fig. 1 E, panels a − c). The time-course well correlated with the development of pathological changes in the lung (Fig. 1 C and E). The XO activity in the sera of MCMV-infected mice was also elevated on day 6 and day 10 (data not shown).

Effect of SOD and allopurinol

The above results suggest that superoxide radicals produced by XO may be involved in the pathogenesis of MCMV pneumonitis in our mouse model. We
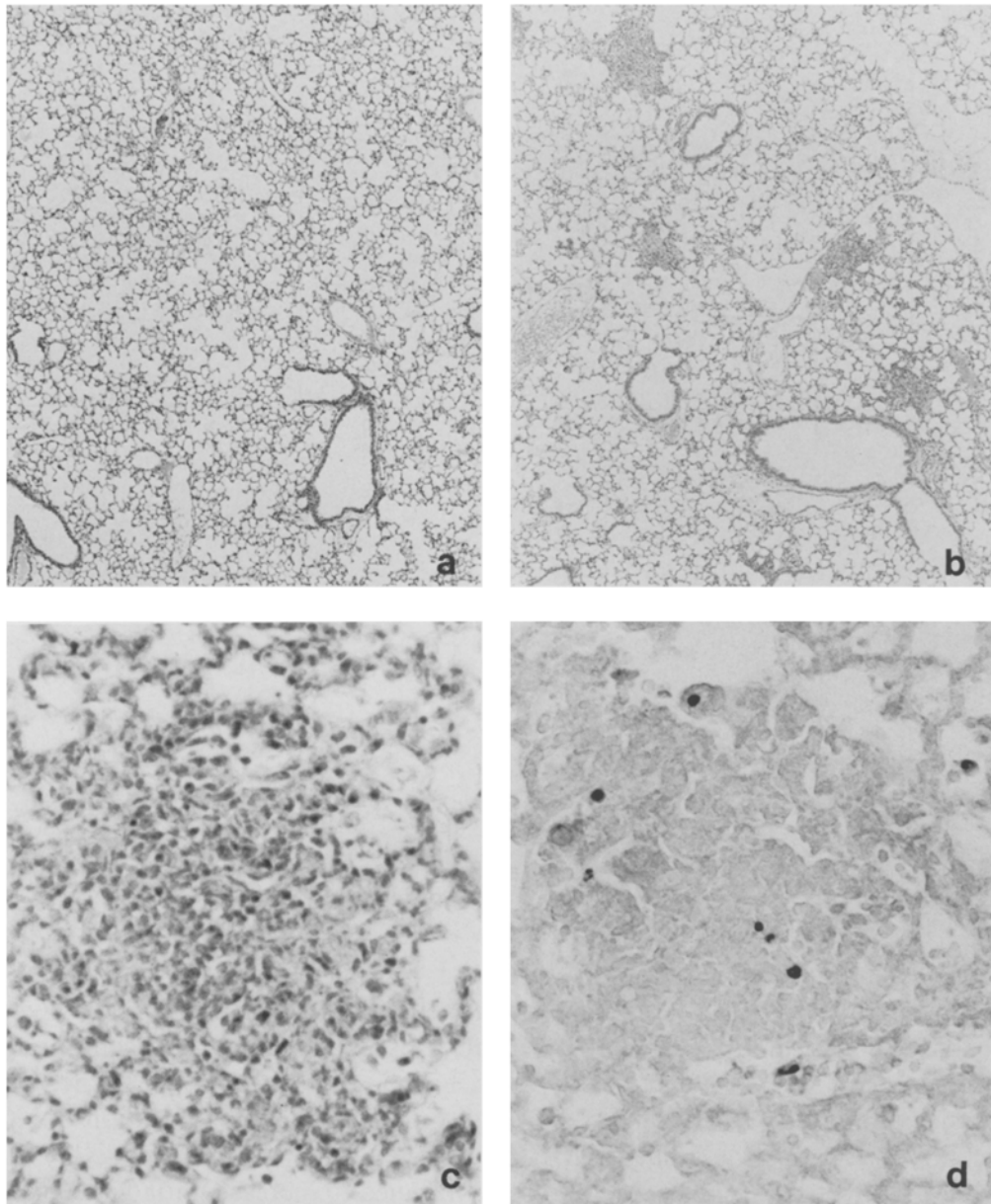
Fig. 2. Histopathology of the lung after intranasal MCMV infection of ICR mice. **a** Lung of mock-infected mice 6 days after infection. No histopathological abnormalities can be seen (H & E, × 40). **b** Lung of MCMV-infected CP 1 mice 6 days after infection. A focal interstitial pneumonitis is evident (H & E, × 40). **c** Inflammatory focus in the lung of MCMV-infected CP 1 mice 6 days after infection. The focus consisted of mononuclear cell infiltration with nuclear debris and fibrin (H & E, × 200). **d** Same microscopic field as in **c**, stained for MCMV antigen using immunoperoxidase technique. Dark reaction products are present in the inflammatory focus (× 200)
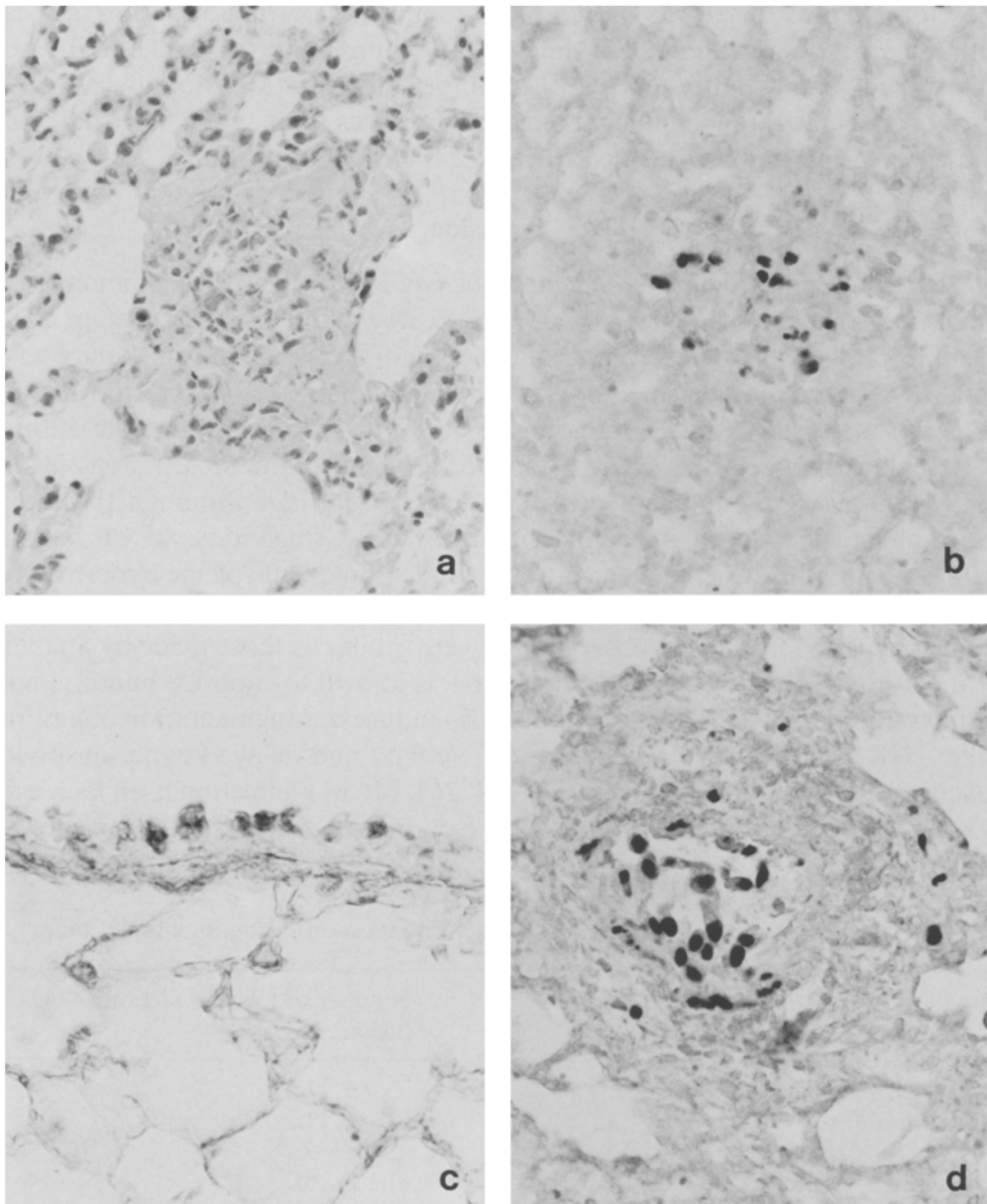
Fig. 3. Histopathology of the lung from MCMV-infected CPn mice 10 days after infection. **a** Inflammatory focus in the lung. The focus consisted of smaller numbers of inflammatory cells and more abundant fibrin than those shown in Fig. 2 c (H & E, × 200). **b** Inflammatory focus stained for MCMV antigen using immunoperoxidase technique. More numerous reaction products per focus were seen when compared with the focus of MCMV-infected CP 1 mice (Fig. 2 d) (× 200). **c** Bronchial epithelial cells stained for MCMV antigen using immunoperoxidase technique. Reaction products were frequently seen in bronchial epithelial cells which were not positive in MCMV-infected mice or MCMV-infected CP 1 mice (× 200). **d** Endothelial cells stained for MCMV antigen using immunoperoxidase technique. Reaction products are evident in endothelial cells (× 200)

therefore studied the effects of SOD and allopurinol on the formation of focal inflammatory lesions in MCMV-infected CP 1 mice, and found that SOD administration significantly reduced the number of foci in MCMV-infected CP 1 mice (Table 1) without significant reduction of virus titers in the lung (data not shown). Allopurinol also tended to reduce the number of foci.

Discussion

We have investigated the pathogenesis of MCMV-associated pneumonitis in immunocompetent ICR mice and in mice treated with the immunosuppressive agent, cyclophosphamide. Intranasal infection of immunocompetent mice with MCMV resulted in transient and self-limited pulmonary lesions which consisted of peribronchiolar foci with mononuclear cell infiltration into the interstitium of alveolar septae. In the MCMV-infected CP 1 mice, the focal lesions in the lung were markedly augmented in their number and size although there was no significant enhancement of the virus growth. A single dose of CP administration caused transient splenic atrophy and subsequent splenic hypertrophy, and the increase in the number of focal lesions coincided with the period of splenic hypertrophy. These observations were similar to those made by Shanley et al. with BALB/c mice [27]. Although CP is known to suppress humoral and cellular immunity [29, 34], the drug can also induce the augmentation of natural killer (NK) cell activity, cytotoxic T cell activity and delayed hypersensitivity reactions under certain conditions [11, 24, 26]. MCMV infection itself increases NK cell activity in the early period of infection and augments cytotoxic T cell

Table 1. Effect of allopurinol and superoxide dismutase on histopathology of lungs[a]

Drug	Dose (/body/day)	Number of foci per section ($\times$ 40) (mean $\pm$ SE)
Saline (i.p.)	—	75.0 $\pm$ 11.2
Allopurinol (i.p.)	0.2 mg[b]	53.4 $\pm$ 11.2
	2 mg	42.9 $\pm$ 9.7[d]
Saline (i.v.)	—	61.1 $\pm$ 6.2
SOD (i.v.)	4000 U[c]	28.8 $\pm$ 4.3[e]

[a] Mice were administrated 200 mg/kg of CP one day before infection, intranasally infected with 7 $\times$ 10[4] PFU of MCMV, and treated either allopurinol or SOD. Drug treatment was started at 24 h after infection. At 6 days after infection, mice were sacrificed and lungs were removed for the histological examination. The histologic evaluation of lungs was done by counting the number of inflammatory foci

[b] Allopurinol dissolved in 0.2 ml of normal saline was given intraperitoneally every 24 h. Control mice were treated with vehicle (normal saline) in the same manner

[c] 2000 U of SOD dissolved in 0.2 ml of normal saline was given intravenously every 12 h. Control mice were treated with vehicle in the same manner

[d] p = 0.051 compared with saline control. n = 7

[e] p < 0.01 compared with saline control. n = 8

activity in the later period [20, 21]. In addition, it has been shown that NK cells are involved in the pathogenesis of MCMV interstitial pneumonitis [22]. Therefore, the enhanced pathological changes observed in MCMV-infected CP 1 mice could be due to augmented NK cell and cytotoxic T cell activities against MCMV infection. On the other hand, a marked increase in the number of foci was also observed in MCMV-infected CPn mice, but histopathological features of the lesions were different from those observed in MCMV-infected CP 1 mice. The foci in the CPn mice were characterized by lesser extent of mononuclear cell infiltration and much more content of fibrin. Since the spleens of CPn mice remained atrophic, the immunological potential is considered to be kept suppressed during the period of observation. Thus it seems that severe pneumonitis in MCMV-infected CPn mice was the consequence of enhanced virus replication in the lung rather than augmented immune response.

Recent studies have shown that oxygen-derived free radicals may play important roles in the pathogenesis of pulmonary diseases associated with inflammation, including pulmonary O_2 toxicity [5], the adult respiratory distress syndrome (ARDS) [9, 31], pulmonary emphysema [8] and idiopathic pulmonary fibrosis [7, 30]. It has been further demonstrated that the activity of XO, which generates superoxide radicals, increases in plasma of ARDS [12] and in the serum and lung of influenza-associated pneumonitis [1, 19], suggesting that XO mediates lung injury through the production of toxic oxygen metabolites. In the present study, we found that XO activity in the lung significantly increased after intranasal infection with MCMV, and that there was a good correlation between the elevation of XO activity and the development of pathological changes in lung tissues. Moreover, allopurinol, a specific inhibitor of XO, and SOD, a specific superoxide radical scavenger were found to reduce the number of the focal lesions in MCMV-infected CP 1 mice. These results suggest that superoxide radicals are involved in the pathogenesis of MCMV-associated pneumonitis in ICR mice. Viral infection itself or host immune response to virus-infected cells may raise the level of superoxide radicals in the microenvironment around the infected cells, probably by accelerating the conversion of xanthine dehydrogenase to XO. Since superoxide radicals can directly cause cellular injury [28] and also act to produce chemotactic substances that attract additional leukocytes to the site of inflammation [18], a rise of the level of the superoxide radicals in the microenvironment could induce further production of superoxide radicals by the secondary cellular injury. Although the precise role of superoxide radicals in the pathogenesis of MCMV pneumonitis is still unknown, such a process seems to be involved in the formation of pulmonary lesions in MCMV-infected mice.

Oxygen free radicals including superoxide radicals are highly reactive, short lived and exist only at low concentrations, and hence their detection in vivo is generally very difficult. Up to now, we have been unsuccessful in identifying the cells or the sites producing superoxide radicals. However, it is known that metabolically activated inflammatory cells release a considerable amount of the

radicals into the suspending medium [4] and the level of XO activity increases in activated leukocytes and macrophages [33]. Besides, capillary endothelial cells, which are a common target of CMV infection, are shown to contain xanthine dehydrogenase in high concentrations [15]. These cells could be cogent candidates for superoxide-producing cells. Further studies will be required to clarify this point.

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References

1. Akaike T, Ando M, Oda T, Doi T, Ijiri S, Araki S, Maeda H (1990) Dependence on O_2^- generation by xanthine oxidase of pathogenesis of influenza virus infection in mice. J Clin Invest 85: 739–745
2. Appelbaum FR, Meyers JD, Fefer A, Flournoy N, Cheever MA, Greenberg PD, Hackman R, Thomas ED (1982) Nonbacterial nonfungal pneumonia following marrow transplantation in 100 identical twins. Transplantation 33: 265–268
3. Avis PG, Bergel F, Bray RC (1955) Cellular constituents. The chemistry of xanthine oxidase. Part I. The preparation of a crystalline xanthine oxidase from cow's milk. J Chem Soc 1100–1105
4. Babior BM, Kipnes RS, Curnutte JT (1973) Biologic defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent. J Clin Invest 52: 741–744
5. Barry BE, Crapo JD (1985) Patterns of accumulation of platelets and neutrophils in rat lungs during exposure to 100% and 85% oxygen. Am Rev Respir Dis 132: 548–555
6. Brody AR, Craighead JE (1974) Pathogenesis of pulmonary cytomegalovirus infection in immunosuppressed mice. J Infect Dis 129: 677–689
7. Cantin AM, North SL, Fells GA, Hubbard RC, Crystal RG (1987) Oxidant-mediated epithelial cell injury in idiopathic pulmonary fibrosis. J Clin Invest 79: 1665–1673
8. Carp H, Janoff A (1978) Possible mechanisms of emphysema in smokers. In vitro suppression of serum elastase-inhibitory capacity by fresh cigarette smoke and its prevention by antioxidants. Am Rev Respir Dis 118: 617–621
9. Cochrane CG, Spragg R, Revak SD (1983) Pathogenesis of the adult respiratory distress syndrome. Evidence of oxidant activity in bronchoalveolar lavage fluid. J Clin Invest 71: 754–761
10. Emanuel D, Cunningham I, Jules-Elysee K, Brochstein JA, Kernan NA, Laver J, Stover D, White DA, Fels A, Polsky B, Castro-Malaspina H, Peppard JR, Bartus P, Hammerling U, O'Reilly RJ (1988) Cytomegalovirus pneumonia after bone marrow transplantation successfully treated with the combination of ganciclovir and high-dose intravenous immune globulin. Ann Intern Med 109: 777–782
11. Gill HK, Liew FY (1978) Regulation of delayed-type hypersensitivity III. Effect of cyclophosphamide on the suppressor cells for delayed-type hypersensitivity to sheep erythrocytes in mice. Eur J Immunol 8: 172–176
12. Grum CM, Ragsdale RA, Ketai LH, Simon RH (1987) Plasma xanthine oxidase activity in patients with adult respiratory distress syndrome. J Crit Care 2: 22–26

13. Grundy JE, Shanley JD, Griffiths PD (1987) Is cytomegalovirus interstitial pneumonitis in transplant recipients an immunopathological condition? Lancet ii: 996–999
14. Hurd J, Heath RB (1975) Effect of cyclophosphamide on infections in mice caused by virulent and avirulent strains of influenza virus. Infect Immun 11: 886–889
15. Jarasch E-D, Bruder G, Heid HW (1986) Significance of xanthine oxidase in capillary endothelial cells. Acta Physiol Scand [Suppl] 548: 39–46
16. Jordan MC (1978) Interstitial pneumonia and subclinical infection after intranasal inoculation of murine cytomegalovirus. Infect Immun 21: 275–280
17. Massey V, Brumby PE, Komai H, Palmer G (1969) Studies on milk xanthine oxidase. Some spectral and kinetic properties. J Biol Chem 244: 1682–1691
18. McCord JM, Wong K, Stokes SH, Petrone WF, English D (1980) Superoxide and inflammation: a mechanism for the anti-inflammatory activity of superoxide dismutase. Acta Physiol Scand [Suppl] 492: 25–29
19. Oda T, Akaike T, Hamamoto T, Suzuki F, Hirano T, Maeda H (1989) Oxygen radicals in influenza-induced pathogenesis and treatment with pyran polymer-conjugated SOD. Science 244: 974–976
20. Quinnan GV, Manischewitz JE (1979) The role of natural killer cells and antibody-dependent cell-mediated cytotoxicity during murine cytomegalovirus infection. J Exp Med 150: 1549–1554
21. Quinnan GV, Manischewitz JE, Ennis FA (1980) Role of cytotoxic T lymphocytes in murine cytomegalovirus infection. J Gen Virol 47: 503–508
22. Quinnan GV, Manischewitz JE, Kirmani N (1982) Involvement of natural killer cells in the pathogenesis of murine cytomegalovirus interstitial pneumonitis and the immune response to infection. J Gen Virol 58: 173–180
23. Reed EC, Bowden RA, Dandliker PS, Lilleby KE, Meyers JD (1988) Treatment of cytomegalovirus pneumonia with ganciclovir and intravenous cytomegalovirus immunoglobulin in patients with bone marrow transplants. Ann Intern Med 109: 783–788
24. Riccardi C, Barlozzari T, Santoni A, Herberman RB, Cesarini C (1981) Transfer to cyclophosphamide-treated mice of natural killer (NK) cells and in vivo natural reactivity against tumors. J Immunol 126: 1284–1289
25. Rose RM, Crumpacker C, Waner JL, Brain JD (1982) Murine cytomegalovirus pneumonia. Description of a model and investigation of pathogenesis. Am Rev Respir Dis 125: 568–573
26. Selgrade MK, Daniels MJ, Hu PC, Miller FJ, Graham JA (1982) Effects of immunosuppression with cyclophosphamide on acute murine cytomegalovirus infection and virus-augmented natural killer cell activity. Infect Immun 38: 1046–1055
27. Shanley JD, Pesanti EL, Nugent KM (1982) The pathogenesis of pneumonitis due to murine cytomegalovirus. J Infect Dis 146: 388–396
28. Southorn PA, Powis G (1988) Free radicals in medicine. II. Involvement in human disease. Mayo Clin Proc 63: 390–408
29. Stockman GD, Heim LR, South MA, Trentin JJ (1973) Differential effects of cyclophosphamide on the B and T cell compartments of adult mice. J Immunol 110: 277–282
30. Strausz J, Müller-Quernheim J, Steppling H, Ferlinz R (1990) Oxygen radical production by alveolar inflammatory cells in idiopathic pulmonary fibrosis. Am Rev Respir Dis 141: 124–128
31. Tate RM, Repine JE (1983) Neutrophils and the adult respiratory distress syndrome. Am Rev Respir Dis 128: 552–559
32. Tsutsui Y, Naruse I (1987) Murine cytomegalovirus infection of cultured mouse embryos. Am J Pathol 127: 262–270
33. Tubaro E, Lotti B, Santiangeli C, Cavallo G (1979) Xanthine oxidase increase in

polymorphonuclear leucocytes and macrophages in mice in three pathological situations. Biochem Pharmacol 29: 1945–1948
34. Turk JL, Poulter LW (1972) Selective depletion of lymphoid tissue by cyclophosphamide. Clin Exp Immunol 10: 285–296

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